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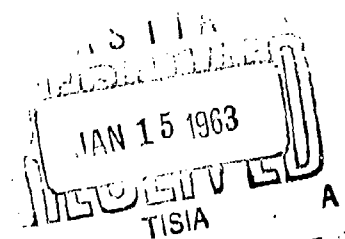
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EXPERIMENTAL INVESTIGATION
OF
DERMACENTOR SILVARUM TICKS
AS CARRIERS OF
VERNAL ENCEPHALITIS VIRUS

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EXPERIMENTAL INVESTIGATION OF DERMACENTOR SILVARUM
TICKS AS CARRIERS OF VERNAL ENCEPHALITIS VIRUS*

- USSR -

[Following is the translation of a report by A. N. Skrynnik and N. V. Ryzhkov, pages 318-320, at the All-Union Conference of Microbiologists and Epidermiologists, January 1939.]

(Department of General Biology and Parasitology and the Department of Microbiology of the Military-Medical Academy imeni S. M. Kirov. According to data of the expedition of Narkomzdrav [Narodnyy Kommissariat Zdravookhraneniya -- People's Commissariat of Public Health] USSR 1937-1938.)

1. Introduction

During the summer of 1937 and 1938, we worked as part of the expeditionary team of Narkomzdrav USSR in one of the vernal encephalitis foci. ([Note:] In 1937, the expedition was headed by Professor L. A. Zil'ber. In 1938, the head of parasitological research was Academician Ye. Pavlovskiy, and of viral research -- Professor A. S. Smorodinetsev). There were three forms of ticks in the region assigned to the northern section of the expedition: *Ixodes persulcatus*, *Haemaphysalis concinna* and *Dermacentor silvarum* (occasionally a fourth form was encountered -- *Haemaphysalis japonica douglasi*).

The object of our research was the *Dermacentor silvarum*. Ticks were gathered from domestic animals and in natural conditions by use of cloth. Out of 1,662 ticks gathered from cows from 10 May to 28 July, 50 percent were *Dermacentor*; 137 ticks from horses were 65 percent *Dermacentor*; 551 ticks from dogs were 0.5 percent *Dermacentor*. Out of 3,000 ticks collected on gauze or removed from people, 8.2 percent were *Dermacentor*.

According to our data, the species composition of ticks in nature varies according to the collection location: some areas yield *Ixodes* almost exclusively, while in others *Dermacentor* prevails. The time of year is no less significant: *Dermacentor silvarum* almost completely disappears by the beginning of July; *Ixodes persulcatus* and *Haemaphysalis concinna* were found in smaller quantities until the very end of our research (in 1937 this was by the middle of August).

* Published in full in the Works of the Military-Medical Academy imeni S. M. Kirov.

2. Duration of viral preservation in adult Dermacentor silvarum

For clarification of the preservation of the vernal encephalitic virus in the tick body, adult ticks were nourished on mice contaminated by virus extracted from man; each group was then subdivided into smaller groups, one of which was nourished on fresh mice for contamination testing. The remaining ticks, after a certain time period, were used in an emulsion for intracerebral administration to fresh mice. Eleven experiments were conducted in all. The emulsion was prepared during the day the ticks were infected, as well as 7 and 28 days following infection. In one of the last cases, the mouse became ill on the tenth day, and later a virus was extracted from it which is still being maintained by successive passaging in mice. Consequently, the virus can endure up to 28 days in the body of a tick that has fed on the blood of an infected mouse; moreover, this is not the maximal time period.

3. Concerning transovarial virus transmission

In order to clarify the possibility of viral transmission from one tick generation to another, we nourished female ticks on infected mice. The offspring of each infected tick was individually separated and tested for contamination by nourishment and intracerebral administration of mice by tick emulsion. We tested the offspring of seven ticks. In one of the experiments a mouse, which had been infested with nymphs from the infected tick on 21 November 1938, became ill on 1 December and died on 2 December 1938. We extracted a virus from the brain of the mouse which was transferred through a number of passages. In this way, transmission of the encephalitic virus from one generation to another was indubitably proven by this experiment.

4. Dermacentor silvarum infection in the larval and nymph stage

In these experiments, tick larva and nymphs were nourished on infected mice, and in the following stages of metamorphosis (nymphs, adult ticks) they were tested by nourishment on fresh mice as well as by the emulsion method. In experiments on infecting larvae, positive results were obtained in three out of five experiments. The infected larvae retained the virus at the nymph stage and through their subsequent transformation into adult ticks. In experiments with nymph contamination, two groups of ticks were tested: one gave a negative, and one a positive, result. Adult ticks, derived from infected larvae and nymphs, infected mice in both test feedings and in the emulsion method test. The possibility of infecting larvae and nymphs by *Dermacentor silvarum* encephalitis virus is very interesting in connection with the question concerning virus reservoirs in nature.

As established by the entomological division of the 1937 Expedition, young stages of *Dermacentor silvarum* in the investigated region are parasites on rodents, chiefly on chipmunks.

The possibility has not been excluded that among ticks taken for our research in experimental infection, we might have gathered ticks naturally infected by encephalitis virus. In experiments with transovarial viral transmission, this is not an essential problem. Larvae from infected ticks were nourished on fresh mice, and nymphs with the virus could only have received it from the parent. As far as the experiments on infecting larvae and nymphs are concerned our conclusions (regarding the possibility and duration of virus retention through tick metamorphosis) stand, regardless of whether we had gathered naturally infected ticks or not. In the very same way, there can be no argument about our proof of the possibility of mice infection through bite from the nymph and adult *Dermacentor silvarum*. We still think, however, that in the majority of experiments yielding positive results, we had experimentally infected the ticks. This is substantiated by the large percentage of successful experiments in virus transmission and the negative results from the parallel research of natural tick infection in the same groups.

A study of the strains obtained through experimental *Dermacentor silvarum* infection showed that the clinical picture observed in mice on intracerebral infection by the indicated strains is identical with the clinical picture produced by the virus directly administered from man. All the strains extracted from the ticks easily pass through Berkefeld's candle V, N, and W. Breeding in nourishing media of brain emulsions of dead mice does not yield future growth either in aerobic or in anaerobic conditions. Experimental infection of experimental mice by isolated strains is possible in other ways: nasally, subcutaneously, intramuscularly, intraperitoneally, intravenously, intracerebrally.

Neutralization attempts, conducted with immune rabbit serum against human virus, yielded positive results. The experiments indicated above leave no doubt that all ten strains, isolated in experimental *Dermacentro silvarum* infection, are identical with the vernal encephalitic virus in humans.

Conclusions

1. In the investigated focus of vernal encephalitis, in addition to other Ixodic ticks, the *Dermacentor silvarum* tick was encountered rather frequently.
2. The possibility has been established that the virus can be retained in the body of experimentally infected adult ticks up to 28 days; moreover this time period is not a maximum one.
3. The possibility has been proven that virus can be transmitted from one generation of ticks to another (transovarial transmission).

4. Ticks infected in the larval and nymphal stage retain the virus for the course of the whole metamorphosis through the adult stage. We observed virus retention for longer than five months, in development from larva to imago.

5. Nymphs and adult ticks, experimentally infected in the preceding metamorphic stage, transmitted the virus by bite.

6. Within experimental conditions, the *Dermacentor silvarum* is a good carrier of the vernal encephalitic virus, and apparently plays a definite role in the circulation of the virus in nature.

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